Initial Review

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In re application of:

David Berd

Serial No.: **08/203,004** Group Art Unit: **1813**

Filed: **February 28, 1994** Examiner: **J. Kresk Staples**

For: **COMPOSITION AND METHOD OF USING TUMOR CELLS**

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

DECLARATION OF DR. DAVID BERD
PURSUANT TO 37 C.F.R. §1.132

I, Dr. David Berd, declare that:

§1 Background

1.1. I am the inventor of the above-identified application directed to a hapten-modified tumor cell composition, useful for the treatment of human tumors.

1.2. I am familiar with the specification and the prosecution history of the above-identified application.

1.3. I am currently a Professor of Medicine in the Department of Medicine, Division of Neoplastic Diseases at Thomas Jefferson University, Philadelphia, PA. A copy of my *curriculum vitae* was attached to the Supplemental Declaration in parent application Serial No. 07/985,334 filed June 23, 1993 (hereinafter Declaration 3). I also authored the Declarations in

grand parent application Serial No. 07/520,649 submitted October 2, 1992 (hereinafter Declaration 2) and March 3, 1992 (hereinafter Declaration 1). A copy of each of the Declarations, together with my *curriculum vitae* is provided herewith.

§2 Discussion of the Present Invention

2.1. The present invention is directed to a composition comprising a hapten conjugated human tumor cell. The composition is typically administered by mixing haptenized cells with an adjuvant and administering the mixture to a patient. To my knowledge, I was the first person to administer hapten conjugated human tumor cells to human patients.

2.2 The composition of the present invention has been clinically tested in humans as discussed in Declarations 1-3.

§3 Discussion of the Prior Art

3.1. Mitchison hypothesized that the pretreatment of animals with a hapten (hapten priming) generates a helper response, whereby T lymphocytes specific for the hapten are generated (hereinafter this theory is referred to as the "Helper Hypothesis"). Mitchison, N. A. *Transplant. Proc.* **11**, 92-103 1970. Researchers believed that the helper response primed the immune system such that subsequent immunization with a hapten-modified antigen enhanced the immune response to the antigen.

3.2. The Helper Hypothesis is the basis of a number of recent approaches to the treatment

of human cancer. For example, cancer vaccines are prepared by genetically modifying cancer cells to produce certain cytokines, such as, interleukin-2 (Saito, S., *et al. Cancer Res.* **1994** *54*: 3516-3520). The cytokine elicits the accumulation of T lymphocytes at the vaccine site which provide "help" to the cancer-specific immune response. However, it is now known that the Helper Hypothesis is not the explanation for successful immunization with hapten-conjugated cells. Recent work from Von Bonin, A., *et al.*, *Int. Immunol.* **1992** *4*:869-874 has shown that the immunogenic component of hapten-conjugated cells consists of hapten-modified, small peptides associated with the major histocompatibility complex (MHC). Of the large number of T lymphocytes that respond to the hapten-modified peptides, a small percentage also recognize unmodified peptides. While not intending to be bound by any particular theory of operation, it is likely that the hapten-conjugated composition of the present invention works by recognizing unmodified peptides.

3.3. Previous to the filing on this and the above-identified related applications, I had been actively involved in the development of cancer vaccines. For example, Berd *et al.*, *Cancer Research* **1986** *46*:2572-2577 disclose the induction of cell mediated immunity to autologous melanoma cells and occasional regression of metastases after administration of cyclophosphamide (CY) followed by treatment with a non-haptenized melanoma cell vaccine. The vaccine used in that study comprised irradiated non-haptenized tumor cells (see page 2573, col. 2, "Preparation of Vaccine and Skin Test Material"). At the time I authored the paper, I believed that a patient had to be pretreated with CY to obtain immunity (see page 2574, col. 2,

"Antitumor Responses - Vaccine Alone").

3.4. The following references also disclose my other attempts to induce immunity against tumors. The list of references is representative of my publications and is not intended to be inclusive. The references describe vaccines which are similar to the one described in Berd *et al.*, *Cancer Research* **1986** *46*:2572-2577 (discussed above) and which also require the coadministration of CY in order to obtain immunity:

Berd *et al.*, *Cancer Research*, **1982**, *42*:4862;

Berd *et al.*, *Cancer Research*, **1984**, *44*:1275;

Berd *et al.*, *Cancer Research*, **1984**, *44*:5439;

Berd & Mastrangelo, *Cancer Research*, **1987**, *47*:3317;

Berd & Mastrangelo, *Cancer Invest.*, **1988**, *6*:337;

Berd *et al.*, *Proc. AACR*, **1988**, *29*:408; and

Berd & Mastrangelo, *Cancer Research*, **1988**, *48*:1671.

3.5 Attempts to treat human cancer using these approaches were unsuccessful. Immunization with autologous non-haptenized melanoma cells failed to reliably induce the development of cell-mediated immunity as indicated by delayed-type hypersensitivity (DTH). This approach did not cause the development of inflammatory responses in metastatic tumor sites, as described for the haptenized composition of the present invention. Finally, a study of autologous non-haptenized vaccine in patients following resection of lymph node metastases produced a negative result: 18/23 patients developed recurrent melanoma and 18/23 died. This

is in contrast to the hapten-conjugated composition of the present invention in which the five-year survival is 58%.

3.6. Other researchers in the field had also proposed cancer treatments.

3.6.a.i. Fujiwara and colleagues published a series of papers on the augmentation of tumor-specific T cell-mediated immunity by "amplifier" T lymphocytes, including:

Fujiwara *et al.*, *J. Immunol.* **1980** *124*:863 (Fujiwara I);

Fujiwara *et al.*, *J. Immunol.* **1984**, *132*:1571 (Fujiwara II); and

Fujiwara *et al.*, *J. Immunol.*, **1984**, *133*:509 (Fujiwara III).

3.6.a.ii. Fujiwara *et al.* disclosed a composition which enhanced immunity following pretreatment with a hapten. Fujiwara's composition comprised a haptenized tumor cell and differed from the present invention in the following respects:

A. The cells used in Fujiwara's composition were derived from induced transplantable murine tumors - not from spontaneous human tumors.

B. Fujiwara's composition is used in immunoprophylaxis - the present invention uses immunotherapy.

C. Fujiwara's composition is administered as a local therapy - the present invention is administered by systemic inoculation.

D. Fujiwara's composition did not result in tumor regression - the composition of the present invention results in regression and 58% of patients treated with the composition of the present invention are surviving.

3.6.a.iii. Fujiwara *et al.* disclose a transplantable mouse tumor which is induced by a carcinogenic agent in one mouse; extracts of that tumor are then injected into other mice. In contrast, the composition of the present invention is derived from human tumors which are spontaneous. As a skilled artisan, I understand that induced tumors are easier to manipulate than spontaneous tumors. That is, induced tumors are easier to treat with immunoprophylaxis and immunotherapeutic methods because they tend to be immunogenic.

3.6.a.iv. In addition, results obtained with induced tumors frequently are not applicable to treatments for spontaneous tumors. This has been repeatedly shown. For example, Hewitt *et al.*, *Br. J. Cancer* **1976** *33*:241-259 describe the differences between spontaneous and induced tumors. He concluded that "practically all the animal data presented in support of a general theory of tumor immunogenicity or to provide a basis for active clinical immunotherapy have been obtained from transplanted tumor systems which entail artefactual immunity associated with viral or chemical induction of the tumors or their allogeneic transplantation." See Hewitt *et al.*, abstract, page 241.

3.6.a.v. Fujiwara *et al.* employ the process of immunoprophylaxis. In this process, a normal mouse is treated with a vaccine. Several weeks later, that mouse is injected with live tumor cells. The growth of the tumor is measured and compared to the growth rate of the same tumor injected into another mouse of the same strain that had not received the vaccine. The haptenized tumor cell composition of the present invention is used in immunotherapy, in which a patient presents with a growing tumor. Only then is the patient treated with a vaccine. While

immunoprophylaxis may more effective than immunotherapy, it is only possible to do immunotherapy in humans.

3.6.a.vi. Fujiwara *et al.* employed trinitrochlorobenzene, TNCB, as a local therapy. TNCB was injected directly into a tumor. In contrast, the present invention uses systemic therapy. The results of Fujiwara *et al.* do not come close to the results achieved with the present invention. In Fujiwara II, Table II, page 1575, 8/11 mice showed tumor regression following a treatment consisting of the combination of a CY injection, TNCB painting (TNP priming) and intratumor injection. However, Fujiwara *et al.* show no tumor regression when the treatment consisted of cyclophosphamide injection and intratumor injection.

3.6.a.vii. In contrast, the composition of the present invention is not used by direct injection into a tumor and does not require TNP priming. At the time of filing Declaration I, 80% of the melanoma patients tested were tumor free 20 months after surgery and treatment with the vaccine of the present invention. Seventy percent of the control group relapsed by 20 months, and all but 5 patients died. See Declaration I, page 4. The 5 year survival data for these patients is now available such that patients treated with the composition of the present invention is 58% compared to 22% for historical controls. In summary, the composition of Fujiwara *et al.* provided no tumor regression whereas the vaccine of the present invention resulted in 58% of patients surviving.

3.6.b. Miller *et al.*, *J. Immunol* **1976** *117*:1519 describe the induction of hapten-specific T cell tolerance by injecting mice with DNP-conjugated normal lymphocytes. Miller *et al.* fail to

administer any tumor cell which is haptenized.

3.7. Even if one assumes that a skilled artisan might have attempted to formulate a composition based on spontaneous human tumors, the present invention provides the surprising results of patients who are tumor free following treatment with the hapten modified tumor cells of the present invention. Many procedures which were successful for treating induced murine tumors have not been successful when applied to spontaneously arising human tumors.

3.8.a. Growth of a transplanted tumor has been prevented by prior immunization with that tumor. For example, Srivastava *et al.*, *Proc. Natl. Acad. Sci.* **1986** *83*:3407 show that mice immunized with inactivated cells from a transplantable carcinogen-induced tumor were able to reject a challenge of live cells from the same tumor. This has not been reproducible with human tumors. For example, attempts have been made to immunize patients with non-haptenized, inactivated melanoma cells, but tumor recurrence after the tumor was resected was unable to be prevented (Berd *et al*, *J. Clin. Oncol.*, **1990** *8*:1858).

3.8.b. Lafreniere and Rosenberg, *J. Immunol.* **1985** *135*:4273, reported regression of hepatic metastases of various tumors in mice by administration of lymphokine-activated killer (LAK) cells administered with interleukin-2. This treatment approach was taken into clinical trials with great enthusiasm, but it has now been abandoned because of poor results and severe

toxicity (Parkinson, D. R., *et al,. J. Clin. Oncol.* **1990** *8*:1650-1656). Also Rosenberg *et al., Science* **1986** *233*:1318, showed that murine tumors could be cured by infusion of so-called tumor-infiltrating lymphocytes (TIL). This approach has not been successful in most human trials and is no longer used (Bukowski *et al., Cancer Res.* **1991** *51*:4199).

§4 Discussion of Enablement

4.1. Haptens

As a skilled artisan, I appreciate that many different haptens may be conjugated to tumor cells to generate an effective composition to treat cancer. A variety of haptens of quite different chemical structure have been shown to induce similar types of immune responses: TNP (Kempkes *et al., J. Immunol.* **1991** *147*:2467); phosphorylcholine (Jang *et al., Eur. J. Immunol.* **1991** *21*:1303); nickel (Pistoor *et al., J. Invest. Dermatol.* **1995** *105*:92); arsenate - Nalefski and Rao, *J. Immunol.* **1993** *150*:3806). To my knowledge, successful conjugation of a hapten to a cell to elicit an immune response is determined by conjugation via ϵ-amino groups of lysine or -COOH groups. This group of haptens include a number of chemically diverse compounds: trinitrobenzenesulfonic acid, fluorescein isothiocyanate, arsenic acid benzene isothiocyanate, trinitrobenzenesulfonic acid, and dinitrobenzene-S-mustard (Nahas and Leskowitz, *Cellular Immunol.* **1980** *54*:241). As a skilled artisan, I expect DNP to be representative of haptens in general for use in the present invention.

4.2. Adjuvants

Several immunological adjuvants have been shown to be equivalent to *Bacillus Calmette-Guerin* (BCG) in experimental and clinical tumor systems. Helling *et al.*, *Cancer Res.*, **1995** *55*:2783, showed that either BCG or the synthetic adjuvant, QS-21, could be used effectively with a ganglioside vaccine in melanoma patients, although QS-21 appeared to be more effective. McCune *et al.*, *Cancer* **1979** *43*:1619, used *Corynebacterium parvum* in place of BCG with a kidney cancer vaccine. Similar results were reported by Bursuker *et al., Int. J. Cancer* **1991** *49*:414, in a murine tumor system. Noguchi *et al.*, *Proc. Natl. Acad. Sci. USA* **1995** *92*: 2219-2223, used interleukin-12 as an adjuvant in a murine tumor immunotherapy system and obtained results similar to those obtained by others with BCG. Diamantstein, *Cancer Res.* **1993** *53*: 714-716, compared *Corynebacterium parvum* with cytokine-transfected tumor vaccines in a murine tumor system and reported similar results. Berd, *Pharmacol Therap A* **1977** *2*:373-395 reviewed the immunology of *Corynebacterium parvum* and showed similarities between that adjuvant and other adjuvants, such as BCG. Thus the preponderance of evidence indicates that many adjuvants have a common mechanism of action. Several adjuvants work as well as BCG in immunotherapy models. Accordingly, BCG is representative of adjuvants in general.

4.3. Other tumor types

4.3.a.i. *Melanoma has the same clinical behavior as other human cancers.* The clinical course of melanoma is distinct in that it begins in the skin. It then metastasizes to regional lymph nodes in a manner similar to other cancers, such as colorectal cancer, breast cancer, and

lung cancer. Eventually melanoma cells enter the blood stream and form distant metastases, particularly in the lungs, liver, and brain. All of these organs are common sites of metastasis for colorectal, breast, and lung cancers (Morton, D.L., *et al.*, Malignant Melanoma. In: J.F. Holland, E.I. Frei, R.C.J. Bast, D.W. Kufe, D.L. Morton and R.R. Weichselbaum (eds.), *Cancer Medicine*, pp. 1793-1824, Philadelphia: Lea and Febiger. **1993**).

4.3.a.ii. The primary conventional treatment for melanoma is surgery, just as it is for colorectal, breast, and lung cancer. When any of these cancer metastasizes to regional lymph nodes, it still may be curable by surgery, but the cure rate is low. Distant metastases are treated by chemotherapy; the drugs used for treatment of melanoma, such as cisplatin, are also used for treatment of the other common cancers. Melanoma is less sensitive to radiation therapy than breast or lung cancer. However, this is not a distinctive characteristic, since other types of human cancer, such as sarcomas, are also relatively radio-resistant (Morton, D.L., *et al.*, Malignant Melanoma. In: J.F. Holland, E.I. Frei, R.C.J. Bast, D.W. Kufe, D.L. Morton and R.R. Weichselbaum (eds.), *Cancer Medicine*, pp. 1793-1824, Philadelphia: Lea and Febiger, **1993**).

4.3.b. *Vaccines have been tested in human cancers other than melanoma although little success has been achieved.* McCune *et al., Cancer* **1981** *47*: 1984-1987, treated patients with adenocarcinoma of the kidney with an autologous vaccine (not haptenized). Tumor regression was seen in a few patients. Schulof, R.S., *et al.*, *Mol. Biother.* **1988** *1*:29-36, obtained positive immunological, although not clinical, results by administering an autologous vaccine (not haptenized) to patient with small cell lung cancer. Kantor *et al., JNCI* **1992** *84*:1084-1091,

showed positive immunological results, but no clinical results, after immunizing colorectal cancer patients with a recombinant vaccine containing carcinoembryonic antigen. Kwak *et al., N. Engl. J. Med.* **1992** *327*:1209-1215, were able to immunize patients with lymphomas to the idiotype expressed by their malignant B cells. Schlag *et al.*, *Cancer Immunol. Immunother.* **1992** *35*:325-330, reported a positive clinical result by administering an autologous (not haptenized), virus-treated vaccine to patients with colorectal cancer. Simons, J.W., *Proc. Am. Assoc. Cancer Res.* **1994** *35*:676-677, has described a strategy to treat prostate cancer with an autologous vaccine (not haptenized) genetically engineered to produce cytokines. MacLean *et al.*, *Cancer Immunol. Immunother.* **1993** *36*:215-222, have reported preliminary positive results of treating breast cancer with a vaccine containing a common antigen. These results show that melanoma is not distinct in its immunogenicity or its potential susceptibility to active immunization. Moreover, the problems with developing successful immunotherapy for human melanoma are the same as for many other types of human cancer.

4.3.c. *Many different murine tumors of varying histological types have been successfully treated with immunotherapy.* These include mastocytoma (Berendt, M.J. and North, R.J., *J. Exp. Med.* **1980** *151*:69-80), plasmacytoma (Hengst, J.C.D., *et al.*, *Cancer Res.* **1980** *40*:2135-2141), sarcoma (Srivastava, P.K., *et al.*, *Proc. Natl. Acad. Sci. USA* **1986** *83*:3407-3411), Lewis lung tumor (Porgador, A., *et al.*, *Cancer Res.* **1992** *52*:3679-3686), colon tumors (Lafreniere, R. and Rosenberg, S.A., *J. Immunol.* **1985** *135*:4273-4280), as well as murine melanoma (Shrayer, D., *et al.*, *Cancer Immunol. Immunother.* **1995** *40*:277-282). Therefore, in murine systems the

results seen with experimental melanomas have been representative of those obtained with other tumor types.

4.3.d. *Many antigens are ubiquitous through many tumor types*

4.3.d.i. MAGE antigen was originally described in human melanomas (Traversari, C., *et al. J. Exp. Med.* **1992** *176*:1453-1457, but is now known to be expressed by breast cancer (Brasseur, F., *et al.*, *Int. J. Cancer* **1992** *52*:839-841; Russo, V., *et al.*, C. *Int. J. Cancer* **1995** *64*:216-221, lung cancer (Weynants, P., *et al.*, *Int. J. Cancer* **1994** *56*:826-829), ovarian cancer (Russo, V., *et al.*, *Int. J. Cancer* **1996** *67*:457-460), gastric cancer (Inoue, H., *et al. Gastroenterology* **1995** *109:*1522-1525), and brain tumors (Rimoldi, D., *et al.*, *Int. J. Cancer* **1993** *54*:527-528.

4.3.d.ii. Proteins encoded by the mutated ras oncogene are potential immunogens. They are found in a variety of human cancers: melanoma (O'Mara, S. M., *et al.*, *Eur. J. Cancer* **1992** *28*:9-11); ovarian carcinoma (Filmus, J. E. and R. N. Buck, *Cancer Res.* **1985** *45*:4468-4472); breast cancer (Lundy, J. R., *et al.*, *J. Clin. Oncol.* **1986** *4*:1321-1325); pancreatic and colon cancer (Qin, H., *et al.*, *Cancer Res.* **1995** *55*:2984-2987).

4.3.d.iii. In addition, many approved drugs work well for many tumor types (Colvin, M., Alkylating agents and platinum antitumor compounds. In J.F. Holland, *et al.* (Eds.), *Cancer Medicine* pp. 743-747, Philadelphia: Lea and Febiger. 1993, and Myers, C., Anthracyclines and DNA intercalators. In J.F. Holland, *et al.* (Eds.), *Cancer Medicine* pp. 743-747, Philadelphia: Lea and Febiger. 1993): daunorubicin - causes regression of breast cancer, lymphoma, leukemias, sarcomas; cisplatin - causes regression of melanoma, lung cancer, and ovarian cancer.

§5

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

§6

Further, deponent saith not

David Berd
Dr. David Berd

11/21/96
Date